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Effect of Dried Orange (*Citrus sinensis*) Pulp on Growth Performance, Serum Biochemical Parameters, and Nutrient Digestibility in Broiler Chickens

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Broiler, Citrus pulp, Liver enzyme, Nutrient, Serum biochemistry, Digestibility

ABSTRACT

This study investigated the effect of dried orange pulp on growth performance, immunity, serum biochemical parameters, and nutrient utilization in broiler chickens. Two hundred and forty straight-run broiler chicks (Cobb 500) were allocated to 4 groups with 5 replicates of 12 chicks each in a completely randomized design. The treatment groups were control (CON), and 3 dried citrus pulps (DCP) e.g., the control group was fed a standard diet/basal diet and the other three groups contained control + 0.5g/kg citrus pulp (DCP-1), control + 1g/kg citrus pulp (DCP-2) and control + 2g/kg citrus pulp (DCP-3) respectively. Results showed a significantly overall higher body weight gain, carcass yield, and better feed conversion ratio (FCR) in the DCP-3 and DCP-2 diet groups as compared to the control and DCP-1 groups. Among serum biochemical parameters, alanine aminotransferase (ALT) in the CON but aspartate aminotransferase (AST) in the CON and DCP-1 diet groups were significantly higher than in the remaining groups. The digestibility of crude protein (CP), ether extract (EE), and apparent metabolizable energy were significantly higher for the DCP-3 and DCP-2 diet groups compared to the control and DCP-1 diet groups. In conclusion, the use of dry citrus pulp improved production performance, liver function, and nutrient digestibility in broiler chicken.

INTRODUCTION

Due to the rising costs of poultry feedstuffs like soybean and cereal grains, it has been observed that the cost of poultry has been rising steadily in recent years. Generally, about 60-80% of expenses in poultry production are related to feeding (Basir and Majid, 2017; Li and Chen, 2019). Cheaper feeding techniques are therefore necessary to reduce the costs associated with animal nutrition when using alternative feeding resources (Pisoschi and Pop, 2015). Since agricultural byproducts make up a larger portion of domestic animals' diets, the use

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of agricultural byproducts in animal nutrition is currently a topic of public concern. The search for new feed sources has become necessary due to a lack of domestic feed resources, particularly in developing nations. As a source of natural antioxidants, dried orange (*Citrus sinensis*) pulp (DCP) has the potential to provide some important nutrients for poultry feed. However, DCP is a typical by-product of citrus juice extraction and is widely produced in Asian agricultural regions. Different scientists reported several types of chemical compounds in *Citrus sinensis* which include volatile oil, flavonoids (e.g., flavanones, flavanone glycosides, and polymethoxylated flavones), carbohydrates, coumarins, peptides, fatty acids, steroids, alkanes, hydroxyamides, carotenoids, carbamates, vitamins and alkylamines (Dongre *et al*., 2023). Globally more than 75.57 million tons of citrus are produced (FAOSTAT, 2022). More than 50% of citrus is used to make juice after the juice is extracted about 60% of the citrus waste is lost which contains valuable antioxidants. After juice extraction from citrus fruits, the leftovers are dried and the pulp is then produced. It is a combination of the peel, the interior sections, and the removed citrus fruits that contain biologically active substances (i.e., flavonoids), which have favorable and helpful effects on the human body (Bampidis, and Robinson, 2006). Fruit and vegetable wastes can be added to animal feeds to increase the diet's palatability, which could increase feed consumption and lower feed costs (Chaudry *et al*., 2004). Citrus pulp, albedo, membranes, and pith contain flavonoids (hesperidin, naringin, etc.), a class of secondary plant phenolic with significant antioxidant and chelating properties (Kamboh *et al*., 2015). Broiler diets already include citrus pulp and its flavonoids, with varying results in terms of growth performance metrics but positive effects on the antioxidant properties of the meat. After the dietary inclusion of dried *Citrus sinensis* peels at the levels of 1.5 or 3.0%, no effect was seen on the final weight, the hot carcass weight, or the carcass yield (Ebrahimi *et al*., 2013). Feed intake and body weight gain were improved when dried orange residues at 2% or dried tangerine peel extract at 80–480 mg/kg were used (Abbasi *et al*., 2015; Jiang *et al*., 2016). Studies have shown that including citrus pulp in broiler diets results in improvements in growth performance metrics etc. (Huyghebaert *et al*., 2011), and this is a stimulus for the implementation of research activities in this direction. The objective of this study was to test and investigate the possible effects of the dietary orange pulp as supplements incorporated into broiler diets on growth performances, serum biochemistry, and nutrient utilization in broiler chickens.

MATERIALS AND METHODS

Bird's husbandry and treatments

Two hundred and forty day-old mixed sex broiler chicks (Cobb 500) were purchased from a local hatchery, weighed individually on arrival, and randomly allocated to 4 treatments and 5 replicates of 12 chicks each in a completely randomized design and reared in the open-sided house for 35 days. The environmental conditions and management practices were per the standard Cobb guidelines. Feed in mash form and water were provided *ad libitum*. The treatment groups were offered a starter (at 1 day to 21 days of age) and a finisher diet (at 22 to 35 days of age). One of the treatment groups was offered the diet with no additive and it served as a control (CON), whereas the other three treatment groups e.g., dried citrus pulp (DCP-1, DCP-2, and DCP-3) were offered diets further supplemented with dried orange pulp, at 0.5 g/kg, 1g/kg and 2g/kg, respectively. The treatment diets were isocaloric and isonitrogenous. All chicks were vaccinated as per the recommended schedule for broilers. Chicks were housed in wire floor pens covered with paper rolls and had free access to mash feed and water throughout the trial. Experimental diets were formulated to provide more or exceed nutritional requirements for broiler chickens based on the Cobb Broiler Manual. The ambient temperature was gradually decreased from 33 to 25°C on day 21 and was then kept constant. Ingredients and nutrient specifications of experimental diets are shown in Table I. All experimental procedures were evaluated and approved by the Ethics Committee Animal Care of the University of Agriculture, Peshawar, Pakistan.

Performance parameters

Body weight (BW) and feed intake (FI) were recorded weekly for each replicate using a digital weighing scale with a measurement accuracy of two decimal points. Data recorded on weekly BW and FI were used to calculate the feed conversion ratio (FCR) (Islam *et al*., 2022).

Carcass characteristics and organ weight

On day 35 of the experiment, two birds close to the mean body weight of the pen were selected, individually weighed, and euthanized. Liver, gizzard, pancreas, and cecum were collected, weighed, and expressed as a percentage of live BW on day 35.

Serum biochemical parameters analysis

At the end of the study on day 35, four birds from each group were randomly selected and fasted overnight. Blood samples (1mL/bird) were collected into EDTA tubes from the wing veins. Samples were transferred to the

Table I. Experimental diet composition and calculated nutrients during the starter and finisher periods.

Ingredient %	Starter phase (1-21days)	Finisher phase (22-35days)
Corn	58.78	63.18
Soybean meal (46 %)	34.47	29.63
Vegetable oil	2.590	3.830
Sodium chloride	0.370	0.370
Limestone	1.470	1.360
Di calcium phosphate	1.080	0.730
Lysine sulfate	0.370	0.220
DL methonoine	0.300	0.240
Threonine	0.110	0.010
Choline chloride (70 %)	0.100	0.100
Mineral and vitamin premix[1]	0.250	0.220
Phytase	0.010	0.010
Calculated nutrients		
Dry matter	84.60	83.35
Crude protein	20.95	18.78
Ash	5.349	4.726
Ether extract	5.450	6.735
Crude fiber	2.791	2.674
AME (Kcal/Kg)	2975	3100
Sodium	0.180	0.180
Chlorine	0.289	0.290
Potassium	0.885	0.799
Calcium	0.900	0.760
Ava. Phosphorus	0.450	0.38
Lysine	1.220	1.020
Methionine	0.597	0.507
Met+Cys	0.910	0.800
Tryptophan	0.227	0.202

[1.]Vitamin A, 5000 IU/g; Vitamin D3, 3000 IU/g; Vitamin E, 80 mg/ g; Vitamin K3, 1.5 mg/g; Vitamin B2, 1 mg/g.; Calcium Pantothenate, 4 mg/g; Niacin, 15 mg/g; Vitamin B6, 13 mg/g; Cu, 10 mg/g; Zn, 80 mg/g; Mn, 80 mg/g; Fe, 60 mg/g; AME, Apparent metabolizable energy.

laboratory for analysis within two hours of collection and centrifuged at 3500 rpm for 15 min to harvest the plasma. The harvested plasma was stored in an Eppendorf tube at -20 ^{0}C until assayed. Biochemical analysis was done according to standard protocols using commercial laboratory kits (Sultan *et al*., 2023).

Nutrient digestibility

To calculate apparent total tract nutrient digestibility (ATD) on day 35 of the experiment, representative chicken from each treatment was transferred to metabolic cages. Chromium oxide (Cr_2O_3) was added to the experimental diet as an indigestible marker at the rate of 0.2% for six days. After processing on day 35, the ileal content was collected and stored at −20 ^{o}C for further analyses of nutrient content. Chromium concentrations were determined with a UV absorption spectrophotometer (Shimadzu, UV-1201, Shimadzu, Kyoto, Japan) using the method while ATD was calculated by using the following formula (Islam *et al*., 2022).

$$\text{ATD } (\%) = 100 - \frac{\text{conc. of marker in feed}}{\text{conc. of marker in digesta}} \times \frac{\text{conc. of nutrient in digesta}}{\text{conc. of nutrient in feed}} \times 100$$

RESULTS

The effect of different levels of dried orange (*Citrus sinensis*) pulp on growth performance during the starter period (1-21 days), finisher period (22-35 days), and total period (1-35 days) are presented in Table II. Feed intake during the starter, finisher, and overall phases was not affected among the groups. During the starter phase, significantly higher body weight gain (BWG) and better FCR were recorded in the DCP-3 diet group than in the remaining groups. At the finisher phase, a significantly higher BWG was calculated for DCP-3 and DCP-2 diet groups than the CON and DCP-1 groups while FCR was ($p<0.05$) higher in the DCP-1 diet groups as compared to all other groups. The overall phase showed a ($p<0.05$) higher BWG and better FCR in the DCP-3 and DCP-2 diet groups in comparison to the DCP-1 and CON groups. In addition, during the entire growing period, the better daily body weight gain was related to treatments including gradually increased DCP content during the total period, whereas the lowest gains were achieved in broilers fed with the control diet. The effect of feeding different levels of DCP on carcass yield and organ weight is presented in Table III. A significantly higher dressing percentage was observed in DCP-2, and DCP-3 diet groups as compared to the control and DCP-1 groups. Relative weights of gizzard, liver, pancreas, and cecum were not affected by the treatments during the entire experimental period. The effect of different levels of DCP on blood parameters is presented in Table IV. The concentration of the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) decreased in groups DCP-2, and DCP-3 as compared to the control group. Concentrations of serum total protein and glucose were not affected ($p<0.05$). The results regarding the nutrient digestibility and AME are presented in Table V. The digestibility of crude protein, ether extract, and AME were recorded ($p<0.05$) higher for the DCP-3 and DCP-2 diet group as compared to the control and DCP-1 diet groups, while the digestibility of dry matter, crude fiber, and ash was not effected ($p>0.05$).

Table II. Effect of graded level of dried citrus pulp (*Citrus sinensis*) on broiler production parameters.

Production traits		Treatments				P value
		CON	DCP-1	DCP-2	DCP-3	
Starter phase (d 1-21)	BWG (g)	688.0±0.63[c]	736.6±0.83[b]	735.2± 0.53[b]	762.2± 0.32[a]	0.000
	FI (g)	1132±0.07	1134±0.01	1122±0.82	1109±0.16	0.692
	FCR	1.56±0.02[a]	1.51±0.04[a]	1.53±0.049[a]	1.49±0.03[b]	0.049
Finisher phase (d 22-35)	BWG (g)	1068.8±0.59[b]	1060±0.82[b]	1089.6±0.59[a]	1076±0.54[a]	0.006
	FI (g)	2116±0.43	2114±0.53	2096±0.20	2135±0.05	0.321
	FCR	1.97± 0.02[b]	2.03±0.03[a]	1.92±0.03[b]	1.98±0.03[b]	0.007
Overall phase (d 1-35)	BWG (g)	1757±0.09[b]	1777±0.79[b]	1825± 0.09[a]	1838±0.01[a]	0.000
	FI (g)	3248±0.40	3248±0.27	3218±0.06	3245±0.65	0.358
	FCR	1.84±0.01[a]	1.82±0.02[a]	1.76±0.02[b]	1.76± 0.01[b]	0.000

Different superscripts along the row indicate significant difference (p <0.05). CON, basal diet; DCP-1, basal diet + 0.5g of dried citrus pulp/kg diet; DCP-2, basal diet + 1g of dried citrus pulp/kg diet; DCP-3, basal diet + 1.5g of dried citrus pulp/kg diet. BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

Table III. Effect of graded level of dried citrus pulp (*Citrus sinensis*) on broiler carcass trait and organ weight.

Traits (%)	Treatments				P value
	CON	DCP-1	DCP-2	DCP-3	
Carcass yield	65.5±0.21[c]	68.8±0.24[b]	71.6±0.22[a]	71.7±0.45[a]	0.002
Gizzard	1.14± 0.01	1.13±0.01	1.15±0.05	1.17±0.01	0.246
Pancreas	0.28±0.03	0.27±0.05	0.26±0.01	0.28±0.02	0.668
Liver	1.76±0.03	1.74±0.03	1.76±0.03	1.76±0.03	0.947
Cecum	0.57±0.02	0.56±0.01	0.56±0.09	0.53± 0.01	0.236

Different superscripts along the row indicate significant difference (p <0.05). For composition of feed for different group, see Table II.

Table IV. Effect of graded level of dried citrus pulp (*Citrus sinensis*) on blood metabolite at day-35.

Liver enzyme test	CON	DCP-1	DCP-2	DCP-3	P value
ALT (U/I)	42.0±0.40 [a]	21.5±0.64[bc]	26.0±0.40[b]	20.5±0.64[c]	0.00
AST (U/I)	30.25±0.47[a]	29.7±0.47[a]	25.0±0.40[b]	25.5±0.64[b]	0.00
Glucose (mg/dl)	255.5±4.31	256.3±4.21	255.3±4.32	256.2±4.01	0.99
Total Protein (mg/dl)	2.77±0.22	2.78±0.20	2.77±0.23	2.79±0.24	0.79

Different superscripts along the row indicate significant difference (p <0.05). For composition of feed for different group, see Table II. ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.

Table V. Effect of graded level of dried citrus pulp (*Citrus sinensis*) on nutrients digestibility and apparent metabolizable energy at day-35.

Nutrient (%)	CON	DCP-1	DCP-2	DCP-3	P-value
Crude fiber	70.82±0.65	70.79±0.50	72.47±0.32	72.70±0.64	0.07
Crude protein	77.96±0.79[b]	77.37±0.46[b]	78.81±0.31[a]	79.18±0.58[a]	0.04
Dry matter	67.88±0.38	68.19±0.42	68.45±0.75	69.71±0.63	0.06
Ash	67.71+0.23	68.86+0.05	67.77+0.15	68.81+0.08	0.71
Ether extract	73.75±0.01[b]	75.82±0.19[b]	78.92±0.08[a]	79.27±0.30[a]	0.00
AME (Kcal/Kg)	2751±1.10[b]	2791±1.22[b]	2831.4±1.93[a]	2883±2.48[a]	0.00

Different superscripts along the row indicate significant difference (p <0.05). For composition of feed for different group, see Table II.

DISCUSSION

In the present study growth performance parameters like body weight gain and feed conversion ratio were improved by the supplementation of dried citrus pulp in the broiler diet. The current study's findings are in agreement with those of Abbasi *et al.* (2015), who found that supplementing broiler chicken feed with 1.5% dried sweet orange peel promoted feed intake and weight gain in 1-21 days after hatch. Similarly, Faiz *et al.* (2017) observed improved production performance in broiler chicken supplemented with dried citrus pulp. The citrus pulp contains compounds with antibiotic-like properties that can reduce the population of harmful bacteria in the intestine; thereby improving overall gut health and potentially leading to better weight gain (Nannapaneni *et al*., 2008). The citrus pulp contains high levels of phenolic compounds, flavonoids, and limonoids, which have antioxidant properties that protect against oxidation in the aqueous medium of the cell (Vlaicu *et al*., 2020; Klimczak *et al*., 2007) and these compounds have been shown to have antioxidant properties, meaning they can help neutralize harmful free radicals and protect cells from oxidative damage (Jayaprakasha and Patil, 2007). Among carcass characteristics, dressing parentage was significantly improved by the supplementation of citrus pulp in the broiler diet. Serum biochemical parameters can provide important information about a bird's overall health status, including its immune function, organ function, and metabolic state. The use of *Citrus sinensis* in different concentrations yielded highly significant results for both liver enzymes i.e., ALT and AST and these results are in line of study with the findings of Abbasi *et al.* (2015), who conducted research on the use of dried sweet orange and its effect on different parameters including ALT and AST and found that sweet orange significantly affect the levels of AST and ALT in broilers. Similarly, Mostafa *et al.* (2016) pointed out in a study that the use of different oils obtained from citrus waste significantly increases the levels of ALT but decreases the levels of other liver enzymes. Moreover, Behera (2020) concluded in a study that the use of 5% citrus waste significantly reduces the levels of ALT and AST in broilers. Also, Oluremi *et al.* (2019) reported a significant effect of citrus pulp on the levels of AST and ALT in broilers. The citrus pulp contains high levels of phenolic compounds, flavonoids, and limonoids, which have antioxidant properties that protect against oxidation in the aqueous medium of the cell (Hong *et al*., 2012; Klimczak *et al*., 2007). These compounds have been shown to have antioxidant properties, meaning they can help neutralize harmful free radicals and protect cells from oxidative damage (Jayaprakasha and Patil, 2007). The supplementation of citrus pulp at different levels had a significant effect on the digestibility of CP and EE compared to the control group. The use of *Citrus sinensis* in different concentrations yielded non-significant results for the different sub-parameters of nutrient digestibility and these results are in line of study with the findings of Christaki *et al.* (2011), who stated that 6% digestible crude protein has no impact on the performance of quail. Similarly, Ndelekwute and Enyenihi (2017) concluded significant results for EE and protein digestibility in broilers fed with lime juice but no significant effect on the dry matter, crude fiber, and ash levels of broilers. Similarly, Oluremi *et al.* (2006) demonstrated that citrus peel @ 30% could be used as an alternative source against corn. Ndelekwute and Enyenihi (2017) concluded significant results for ether extract and protein digestibility in broilers fed with lime juice but no significant effect on the dry matter, crude fiber, and ash levels of broilers. Moreover, Silva *et al.* (2013) reported that carbohydrates obtained from orange pulp especially pectin has a significant effect on the digestibility of broilers both in the starter and finisher phase. Similarly, Amaga *et al.* (2019) stated a significant effect of dried sweet orange pulp on the nutrient digestibility in broilers used at different levels. Silva *et al.* (2013) also checked the effect of pectin extracted from citrus pulp on the different digestibility parameters and the results revealed that pectin increased the intestinal viscosity while decreasing the excreta moisture content along with a significant change recorded in the levels of apparent metabolizable energy. Oluremi *et al.* (2019) studied the effect of dried sweet orange pulp on different digestibility and blood serum parameters and the results showed high levels of metabolizable energy which ultimately led to the suggestion of the use of sweet orange pulp as a source of energy. The slight variation in this research seems by chance and there is very limited data available regarding the use of sweet orange pulp in the literature. There may be multiple reasons for this variation, which include the sex, age, and heredity of the birds while the source of the sweet orange pulp may also contribute to this change. The higher crude fiber concentration in the pulp may determine the low performance of broilers.

CONCLUSION

Dietary supplementation of dried orange (*Citrus sinensis*) pulp has a useful effect on growth performance, ileal nutrient digestibility, and liver health in broilers and can be used in broiler diets up to 1.5g/kg feed without a negative effect on overall performance, nutrient digestibility, and liver health.

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IRB approval

The departmental board of studies approved the experimental protocols (Approval number: 189-2022/PS/UAP.

Ethical approval

The ethical committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar, Pakistan approved all the experimental procedures adopted.

Statement of conflict of interest

The authors have declared no conflict of interest.

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